Abstract:
Our overall research objectives were to (1) assess population trends along San Nicolas Island and in Monterey County; (2) optimize black abalone spawning methods; (3) develop and validate a real-time PCR assay for quantification of RLP loads (infection intensity); and (4) examine if progeny of surviving black abalone along the California islands are more resistant to WS than are animals without this disease pressure. At UCSB we were focused primarily on objective (2). We tested the following hypotheses to fulfill our objectives. Hypothesis 1: Black abalone spawning requires environmental conditions similar to their intertidal and shallow subtidal habitat, and not standard methods that were developed for subtidal species. Hypothesis 2: Quantitative real-time PCR can be used to quantify loads of the WS rickettsial bacterium (infection intensity) in abalone. Hypothesis 3: Juvenile black abalone recruiting along the California Channel Islands are more resistant to WS than are black abalone in northern Central California that have not experienced high disease (WS) selection pressure.

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California Sea Grant Sea Grant
Final Project Progress Report

1/29/2009

R/F-200A
Assessing withering syndrome resistance in California black abalone:
Implications for conservation and restoration
3/01/2006–09/01/2008

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Project Hypotheses
Our overall research objectives were to (1) assess population trends along San Nicolas Island and in Monterey County; (2) optimize black abalone spawning methods; (3) develop and validate a real-time PCR assay for quantification of RLP loads (infection intensity); and (4) examine if progeny of surviving black abalone along the California islands are more resistant to WS than are animals without this disease pressure. At UCSB we were focused primarily on objective (2). We tested the following hypotheses to fulfill our objectives.

Hypothesis 1: Black abalone spawning requires environmental conditions similar to their intertidal and shallow subtidal habitat, and not standard methods that were developed for subtidal species.
Hypothesis 2: Quantitative real-time PCR can be used to quantify loads of the WS rickettsial bacterium (infection intensity) in abalone.
Hypothesis 3: Juvenile black abalone recruiting along the California Channel Islands are more resistant to WS than are black abalone in northern Central California that have not experienced high disease (WS) selection pressure.

Project Goals and Objectives
We have four integrated research objectives: The first objective was to survey existing populations of black abalone to examine survivorship in stocks potentially resistant to WS, and extend a critical population time-series for this species. The second objective was to develop reliable spawning methods for black abalone, a species that has been difficult to spawn in captivity. Spawning black abalone is a critical step in designing field and laboratory experiments designed to test out-plant strategies for potential disease-resistant individuals. Natural populations of black abalone are critically low, so spawning individuals is the only feasible means of obtaining enough individuals for future experiments designed to examine processes that limit population abundance and distribution. We proposed to conduct controlled laboratory studies to identify the most effective and cost-efficient spawning technique. The third objective was to perfect a molecular Q-PCR method of quantifying WS bacterial infections. Lastly, our fourth objective was to examine the potential heritability of WS resistance in black abalone by characterizing the relative resistance of newly recruited black abalones from SNI to those that have been under little or no WS-selection pressure from the Monterey area (Carmel and Sobranes Points). We were confident of obtaining RLP-naòve individuals from Carmel and Sobranes Points because it is very unlikely that these northern

populations have undergone selection for disease resistance based on recent surveys (Friedman and Finley 2003).

Briefly describe project methodology
Abalone were collected from sites near Carmel, California and placed in flow-through laboratory aquaria at UCSB. All holding tanks were equipped with a mechanical wave generator, an artificial tidal cycle, and timed photoperiod and moon light phase.

In southern California, black abalone primarily spawn in summer and early fall. Accordingly, we conducted spawning experiments during this time. A standard method to spawn black abalone was initially tested as follows: Abalone were removed from aquaria and placed in holding tanks containing filtered sea water to minimize levels of Dissolved Inorganic Carbon (DIC) prior to spawning trials. Abalone are removed from holding tanks and dessicated for one hour in a shaded moist environment. Individuals were then submerged in containers receiving seawater irradiated with ultraviolet light at a dosage of 800 mW h/L. After one hour in the seawater bath, seawater temperatures were raised 5-10 degrees C above ambient and allowed to return to ambient. During this last phase, rooms were darkened to simulate the onset of night. A second treatment involved the use of hydrogen peroxide to induce spawning following placement of abalone in UV irradiated sea water baths. Gametes were removed from tanks containing spawned individuals within 30 minutes of spawning

Describe progress and accomplishments toward meeting goals and objectives
From 2006 through 2008, we (UCSB) collected a total of 42 adult broodstock black abalone from sites in the Carmel, CA region. Individuals were taken from 3 different sites that were deemed to have relatively sustainable numbers of animals. In addition to the adult black abalone we collected, 32 more individuals were given to us from the CA Dept of Fish and Game after apprehending them from a poacher in the San Francisco region. All abalone were brought to UCSB and fed an antibiotic (oxytetracycline) feed for 5 days to reduce potential mortality from Withering Syndrome.

Abalone were initially housed in 2-400 gallon flow through aquaria on the campus of UCSB. These tanks are equipped with a two foot tidal cycle, and artificial daylighting, and moonlighting, which are all timed to coincide with natural cycles from the Carmel region. The tanks are also equipped with surge tanks which flush 40 gallons/minute to simulate wave action in the intertidal. Abalone were fed a constant diet of Macrocystis pyrifera, Egregia menziesii, and Gigartina sp. Water was maintained at ambient temperatures between 14 and 17 degrees Celsius. In September 2007, all individuals were moved into 101 gallon flow-through aquaria, also equipped with a two foot tidal cycle, and artificial daylighting and moonlighting timed to coincide with natural cycles from the Carmel region. The 101 gallon tanks were used to better simulate natural hydrodynamic conditions.

The hydrogen peroxide/UV filtered seawater spawning protocol has proved most successful at spawning gravid female black abalone on numerous occasions. Over the course of this study, however, we have seen limited reproductive development in male black abalone and captive spawning efforts have failed. Previous investigations have identified reproductive development as the primary determinant of captive spawning success. This discrepancy in reproductive development among sexes prompted researchers at UCSB to modify objectives to

include a preliminary survey of investigating the ubiquity of this pattern in wild populations of black abalone along the California coast (discussed below).

Project modifications
Limitations to reproductive development in captive male black abalone initiated ongoing, seasonal, coast-wide surveys of this pattern in the field. Known aggregations of black abalone from Carmel south to Santa Cruz Island are sampled at three month intervals, and reproductive development is qualitatively sampled using gonad indices developed by Uki and Kukuchi (1982) and measurements of gonad distance to shell margin. Initial results suggest limitations to reproductive development are widespread across this species' range, however reproduction appears to occur as the recruitment of juvenile black abalone is observed in areas of high adult abundance. This finding suggests patterns of reproductive development in male abalone may not be a predictable as previously identified, and has initiated further research efforts into the reproductive biology and behavior of this species.

Project outcomes
Spawning protocols developed during this investigation have been shared with captive breeding programs for the endangered white abalone, Haliotis sorenseni, and these efforts have seen some success. In addition, the widespread patterns of limitations to reproductive development identified through this work have initiated efforts to identify the mechanisms driving this pattern.

Impacts of project
The results of this project have significantly changed perceptions of the population dynamics of black abalone, in particular, the reproductive ecology of this species. Recently listed as endangered under the federal Endangered Species Act, restoration efforts will require a sufficiently thorough understanding of this species' reproductive potential. Black abalone require dense spawning aggregations to ensure successful fertilization and limitations to reproductive success will further inhibit recovery. The discovery of reproductive limitations has therefore prompted additional research questions investigating the mechanisms of this patterns.

Benefits, commercialization and application of project results
California Department of Fish and Game: Ian Taniguchi (itaniguchi@dfg.ca.gov)
National Oceanic and Atmospheric Association: Melissa Neuman (melissa.neuman@noaa.gov)
Results were used to identify and prioritize southern California abalone species with the greatest need for restoration efforts.
Bodega Marine Lab, University of California Davis: Gary Cherr(gncherr@ucdavis.edu)
Results from this study initiated research efforts to identify mechanisms of reproductive limitations in California abalone species

Economic benefits generated by discovery
The hydrogen peroxide/UV irradiated seawater spawning protocol developed through this investigation has been tested successfully in other California abalone species, benefiting aquaculture efforts across CA.

Issue-based forecast capabilities
Limitations to reproductive development will impact the reproductive potential of wild populations of black abalone, particularly as populations decline. This

research highlights the need to forecast potential declines of black abalone and actively restore or enhance populations prior to collapse.

Tools, technologies and information services developed
The hydrogen peroxide/UV irradiated seawater spawning protocol developed through this investigation has been tested successfully in other California abalone species, including the endangered white abalone, Haliotis sorenseni.

Publications
Peer-reviewed journal articles or book chapters
Title: Captive breeding of black abalone, Haliotis cracherodii, to enhance natural populations in southern California
Authors:Tal Ben-Horin, Jono Wilson, Tom McCormick, Hunter Lenihan
Date:In preparation
Journal Name: Target: Ecological Applications

Media
Name of publication/radio station, etc: *Sea Grant News*
City: La Jolla
State: CA
Date of publication/broadcast: Novermber 5, 2007
Headline or topic: Disease Resistant Black Abalone Discovered

Name of publication/radio station, etc: *Sea Grant News*
City: La Jolla
State: CA
Date of publication/broadcast: June 27, 2008
Headline or topic: Abalone Restoration Update

Workshops/presentations
"Dynamics of population declines of black abalone in the California Channel Islands: Implications for the persistence of remaining populations"
Poster presentation at the Society for Conservation Biology Annual Meeting, Chattanooga, TN by Tal Ben-Horin

"Captive breeding of black abalone, *Haliotis cracherodii*, to enhance natural populations in southern California"
Presentation given to a graduate level Ecological Sustainability seminar at the University of California Santa Barbara by Tal Ben-Horin

"Limitations to reproductive development in wild populations of black abalone, *Haliotis cracherodii*, along the California coast"
Presentation given to the Marine Science Institute at the University of California, Santa Barbara by Hunter Lenihan and Tal Ben-Horin.

"Restoring California abalone"
Presentation to 2008 Luce Conservation Fellowship Seminar series, Marine Science Institute, University of California, Santa Barbara.

Dissemination of results
Lectures in Bren School, University of California Santa Barbara courses (Applied Marine Ecology, Ecology, Fisheries Science, and Restoration Ecology) in winter, spring, and fall school quarters 2007 and 2008.

Students
Tal Ben-Horin
University of California Santa Barbara
Environmental Science and Management
Degree program enrolled in: Ph.D.
Theses/dissertation title: Spatial ecology of black abalone: habitat associations, disease occurrence, and movement behavior
Supported by Sea Grant funds? [X] yes [] no
08/01/2007⇊05/31/2008

Jono R. Wilson
University of California Santa Barbara
Environmental Science and Management
Degree program enrolled in: Ph.D.
Theses/dissertation title: Marine protected areas and the management of nearshore fishes
Supported by Sea Grant funds? [X] yes [] no
03/01/2006⇊07/31/2007

Gabriela) Navas
University of California Santa Barbara
College of Creative Studies
Degree program enrolled in: B.S.
Supported by Sea Grant funds? [] yes [x] no
09/01/2006⇊05/31/2008

How many students volunteers were involved in the project? 3

Cooperating organizations
Federal
National Marine Fisheries Service: facilitation of permits under the Endangered Species Act
United States Navy/Naval Air Warfare Weapons Division: facilitation of the use to San Nicolas Island for collections and demographic surveys

Local and state
California Department of Fish and Game: assisted with permitting

Nongovernmental
Santa Barbara ChannelKeeper: technical advice
Island Conservation Group: technical advice

Industry
The Cultured Abalone: technical advice
Other Sea Grant programs

Academic Institutions
University of California Santa Cruz/Granite Canyon Marine Laboratory and Protected Resources Division: abalone have been housed at this facility during collecting trips

International implications
We developed close working relationships for research with:
Jeremy Prince, Adjunct Associate Professor, Murdoch University
Arturo Ramiraz Valdez, Grupo Ecologia y Conservacion de Islas, Baja, Mexico

Keywords
Black abalone, Haliotis cracherodii, captive breeding, disease resistance